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COMPARISON OF HARDNESS- AND CHLORIDE-REGULATED ACUTE EFFECTS OF SODIUM SULFATE ON TWO FRESHWATER CRUSTACEANS

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Abstract—Based on previous observations that hardness (and potentially chloride) inßuences sodium sulfate toxicity, the objective of the current study was to quantify the inßuence of both chloride and water hardness on acute toxicity to *Hyalella azteca* and *Ceriodaphnia dubia*. In addition, observed toxicity data from the present study were compared to toxicity predictions by the salinity/toxicity relationship (STR) model. Hardness had a strong inßuence on sulfate toxicity that was similar for both crustaceans, and nearly identical median lethal concentration (LC50)/hardness slopes were observed for the two species over the tested range. Chloride had a strong but variable inßuence on sulfate acute toxicity, depending on the species tested and the concentration range. At lower chloride concentrations, LC50s for *H. azteca* strongly were correlated positively with chloride concentration, although chloride did not affect the toxicity of sodium sulfate to *C. dubia*. The opposite trend was observed over the higher range of chloride concentrations where there was a negative correlation between chloride concentration and sulfate LC50 for both species. The widely ranging values for both species and a high correlation between LC50s in terms of sulfate and conductivity suggested that, whether based on sulfate, conductivity, or total dissolved solids (TDS), attempts at water quality standard development should incorporate the fact that water quality parameters such as hardness and chloride strongly inßuence the toxicity of high TDS solutions. The STR model predicted toxicity to *C. dubia* relatively well when chloride was variable and hardness Þxed at approximately 100 mg/L; however, the model did not account for the protective effect of hardness on major ion/TDS toxicity.

Keywords—Sulfate Total dissolved solids *Hyalella* *Ceriodaphnia* Salinity/toxicity relationship model

INTRODUCTION

Currently no federal water quality criteria exist for the protection of freshwater life for total dissolved solids (TDS), sulfate, or sodium; however, toxicity due to major ions or TDS has received increasing attention in recent years [1,2]. Ordinarily benign major ions (e.g., sodium, sulfate) and, therefore, TDS, which is essentially the sum of the concentrations of all common ions (e.g., sodium, potassium, calcium, magnesium, chloride, sulfate, and bicarbonate) in freshwaters, can reach concentrations in wastewater discharges that severely impair sensitive aquatic species [3–7]. Common sources of efßuents with elevated TDS include reverse osmosis systems, pH modiÞcations of wastewater, agricultural runoff, gas and oil production, and coal or metal mining operations [1]; investigations of major ion toxicity also have included inundation of freshwater systems by brackish water and laboratory-formulated salt solutions [5–13].

The fact that TDS toxicity is dependent on the ionic composition of a water or efßuent has been well established [7,9,12,14,15]. Mount et al. [12] developed statistical models to predict toxicity of high TDS waters to standard test organisms based on ionic composition; seven major ions (Na , K , Ca^{2} ,M g , Cl , HCO$_3$, and SO$_4^{2}$) were included in the analysis. In that study, solutions were more toxic when dominated by particular major ions (i.e., K ,M g , HCO$_3$), and toxicity due to several individual ions, including SO$_4^{2}$, to *Ceriodaphnia dubia* and *Daphnia magna* was reduced when solutions contained more than one cation [12]. Several researchers have observed that hardness and/or multiple nontoxic cations in solution ameliorate major ion toxicity [9,12,15], and laboratory experiments with synthesized freshwaters have demonstrated that increasing hardness at a constant calcium-to-magnesium ratio (Ca:Mg) results in decreased sodium sulfate toxicity to *C. dubia* [14].

In experiments with sodium sulfate in laboratory-synthesized freshwaters, Soucek and Kennedy [14] observed that, while composition of dilution water strongly affected sulfate toxicity to *C. dubia*, the magnitude of the effect on *H. azteca* was even greater. SpeciÞcally, whereas the median lethal concentrations (LC50s) for *C. dubia* in two diluents with different chloride concentrations and Ca:Mg ratios ranged from 2,050 to 2,526 mg SO$_4^{2}$ /L, the LC50s for *H. azteca* in the same two diluents were 512 and 2,855 mg SO$_4^{2}$ /L, respectively [14]. Freshwater organisms use several different osmoregulatory strategies, but most freshwater amphipods and daphnid cladocerans regulate hypertonically with respect to the surrounding medium; this is achieved by active transport of ions, principally chloride, into the hemolymph (see [16–19]). However, even among amphipods, there is a wide range of sodium and chloride inßux rates and integument permeabilities, which determine osmoregulatory effectiveness [20,21], so the ionic composition of water may regulate to varying degrees the response of different species to high levels of sodium sulfate.

The observed contrast in responses of *C. dubia* and *H. azteca* to sulfate under different water quality conditions [14] led to the interest in quantifying the relationships between sulfate toxicity and hardness and chloride concentrations for the two distantly related freshwater crustaceans. Therefore, the objectives of the current study Þrst were to determine the inßuence of hardness on sodium sulfate toxicity to *H. azteca* and to compare its responses to those of *C. dubia* and, second, to quantify the effects of chloride on acute toxicity of sodium

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sulfate to *H. azteca* and *C. dubia* over a wide range of chloride concentrations. The data generated in the Þrst two objectives were useful for investigating the relationship between LC50s calculated in terms of sulfate and those calculated in terms of conductivity to determine the potential utility of a conductivity- or TDS-based water quality standard. The data also presented an opportunity to test the effectiveness of the salinity/toxicity relationship (STR) model developed by Mount and Gulley [22] in predicting acute toxicity of a wide range of sodium, sulfate, chloride, and hardness combinations to *C. dubia*.

MATERIALS AND METHODS

General culturing and testing methods

The cladoceran, *Ceriodaphnia dubia*, was cultured in the laboratory according to U.S. Environmental Protection Agency (U.S. EPA) methods [23]. Amphipods, *Hyalella azteca*, also were cultured in the laboratory according to U.S. EPA methods [24] in a reformulated, moderately hard, reconstituted water described in Smith et al. [25].

For toxicity testing, a pure (99%) grade of anhydrous sodium sulfate (Na_2SO_4) (Chemical Abstract Service [CAS] 7757-82-6) was obtained from Fisher ScientiÞc (Pittsburgh, PA, USA) to serve as the source of sulfate. Previous experiments indicated that the salts and deionized water sources used for our experiments had low to undetectable levels of trace metal contaminants [14,26].

For deÞnitive static, nonrenewal toxicity tests, conducted according to American Society for Testing and Materials E729-96 methods [27], treatments comprised a 75% dilution series (i.e., the 100% concentration was diluted serially by 25%), rather than the standard 50%, because major ion toxicity tests often cause 100% mortality in one concentration and 0% mortality in the next highest concentration if the spread is too great. Five to six concentrations were tested in addition to controls with four replicates tested per concentration. Tests with *C. dubia* were conducted for 48 h with a 16:8-h light:dark photoperiod at 25 C, and *H. azteca* were exposed for 96 h at2 2 C and a 16:8-h light:dark photoperiod. Both organisms were exposed in 50-ml glass beakers with Þve organisms per beaker and, for H. *azteca*, 1 g of quartz sand was added to each beaker to serve as substrate. Only one of the 63 tests was fed, and that fed test had an LC50 in the range of two other tests conducted with the same organism in the same water without food. *Ceriodaphnia dubia* used were less than 24-h old, and *H. azteca* were approximately third instar (7–14-d old). Percent survival in each replicate was recorded every 24 h and at the end of the exposure period. A dissecting microscope was used to assess survival of *H. azteca*.

Standard water chemistry characteristics were measured at both the beginning and the end of each exposure period. Temperature, pH, conductivity, and dissolved oxygen were measured using appropriate meters, and alkalinity and hardness were measured (beginning of tests only) by titration as described by American Public Health Association et al. [28]. Samples from each treatment were analyzed to conÞrm sulfate concentrations by ion chromatography at the Illinois Natural History Survey Aquatic Chemistry Laboratory (Champaign, IL, USA).

All LC50s were calculated based on both measured sulfate concentrations and measured speciÞc conductivity values for each test concentration using the Spearman-Karber method [29]. To increase conÞdence in LC50s, three to Þve assays were conducted with each organism for each water quality combination. This provided a stronger estimate of the mean LC50 for a given set of water quality characteristics for each species. In all, 63 LC50s were generated.

Influence of chloride on the toxicity of sodium sulfate

In these experiments, the toxicity of sulfate (with sodium as the major cation) to *H. azteca* and *C. dubia* was measured in freshwater solutions having nominal chloride concentrations of 1.9, 10, 15, 20, 25, 33 (*H. azteca* only), 100, 300, and 500 mg Cl/L. Chloride, as NaCl (CAS 7647-14-5, Fisher ScientiÞc Catalog AC42429-0010), was added at appropriate concentrations to a solution with a hardness of approximately 100 mg/L (molar ratio of Ca:Mg 1.41; 2.33 in terms of mass). The Ca:Mg ratio was chosen because it is the median value for water bodies sampled in Illinois (R. Mosher, Illinois Environmental Protection Agency, SpringÞeld, IL, USA, personal communication). Whole carboys were made for each elevated chloride level, and this water was used as both diluent and control; therefore, each concentration within a given test had the same chloride concentration (i.e., [Cl] did not change with dilution). The only parameters that varied within a particular test were sodium, sulfate, and conductivity. At least three tests were conducted for each hardness to provide a mean LC50 and standard deviation. Exposures were conducted using the same laboratory and calculation methods described above.

After LC50s were calculated as described above, regression analysis was conducted using JMP software [30] to determine the relationship between chloride concentration and sulfate LC50 for each species; mean LC50s for each chloride concentration were used in these analyses. Because observation of data scatter indicated two different trends were involved depending on the chloride concentration range, two separate analyses were conducted for each species: One for the range of 5 (1.9 mg/L nominal concentration) to 25 mg Cl/L and one for the range of 25 to 500 mg Cl/L. Then, multiple regression analysis with covariance was conducted for the same data ranges using all individual data points to generate an equation for both species, and to determine if the curves were signiÞcantly different for the two species.

Influence of hardness on the toxicity of sodium sulfate

In these experiments, the toxicity of sulfate (with sodium as the major cation) to *H. azteca* was tested in six freshwater solutions having nominal hardness values of 100, 200, 300, 400, 500, and 600 mg/L (as $CaCO_3$). Hardness was increased by adding enough $CaSO_4$ (CAS 7778-18-9) and $MgSO_4$ (CAS 7487-88-9), at a set molar ratio (Ca:Mg 1.41; 2.33 in terms of mass), to achieve the nominal hardness. Measured hardness values for all tests were similar to target nominal hardness values (2.2%). A chloride concentration of 25 mg/L was used for all tests investigating the effects of hardness on sodium sulfate toxicity to *H. azteca* based on results from the above-described tests investigating the effects of chloride on sodium sulfate toxicity to *H. azteca* and *C. dubia*. Whole carboys were made for each elevated hardness level, and this water was used as both diluent and control; therefore, each concentration within a given test had the same hardness (i.e., [Ca^{2}] and [Mg^{2}] did not change with dilution). The only parameters that varied within a particular test were sodium, sulfate, and conductivity. At least three tests were conducted for each hardness to provide a mean LC50 and standard deviation. Exposures were conducted using the same laboratory and calculation methods described above. The LC50s for *H. azteca* were compared to previously generated LC50s for *C. dubia* [14], which were conducted in solutions having a Ca:Mg molar ratio of 0.88 and [Cl] of 5 mg/L.

After LC50s were calculated as described above, regression analysis was conducted using JMP software [30] to determine the relationship between hardness and sulfate LC50 for each species. Mean LC50s for each hardness were used in these analyses. Then, multiple regression analysis with covariance was conducted for the same data ranges using all individual data points to generate an equation for both species and to determine if the curves were signiÞcantly different for the two species.

Relationship between sulfate LC50s and conductivity LC50s

To investigate variability in conductivity at sulfate LC50 concentrations, linear regression analysis was used, and *C. dubia* data from Soucek and Kennedy [14] were included in the analyses.

Comparison of test results with STR model predictions

To compare results from the present study to toxicity predictions generated by the STR model [22], nominal concentrations of all constituent ions (except H and OH , which are not required by the model) were calculated at observed mean sulfate LC50s for *C. dubia* in each test solution type (Cl *x*, hardness *y*, and Ca:Mg *z*). The model does not predict toxicity to *H. azteca*. Ions required by the model are Ca^{2} , Mg^{2} ,Na , K ,Cl , HCO_3, and SO_4^{2} . These calculations were possible because adding known salt concentrations to deionized water generated all test solutions. The average of the absolute value of % difference between nominal and measured sulfate concentrations was 2.082%.

The model output includes equivalents of cations and anions and requires that, to have conÞdence in model output, the difference between the two be less than 15% [22]. The average (standard deviation) % difference between cations and anions for inputs was 0.09 (0.01)%, indicating excellent agreement between cation and anion equivalents. Other model outputs included calculated TDS, a NUMCAT value that estimates effective number of cations, LC50 in terms of % of solution, and % survival in 100% of solution. To examine the predictive ability of the STR model over the range of solutions tested, we created scatter plots of predicted % survival versus either chloride concentration or water hardness as appropriate for each species tested. Because all of our input values were ion concentrations calculated at experimentally observed sulfate LC50s, the observed % survival was always 50%. Observed % survival data were plotted as a horizontal line for comparison to STR-predicted data.

RESULTS

Influence of chloride on the toxicity of sodium sulfate

Chloride had variable effects on sodium sulfate toxicity to *C. dubia* and *H. azteca* over the range of 5 to 500 mg Cl /L. For *H. azteca*, two different linear trends were observed depending on the chloride range (Fig. 1A and B). Increasing chloride concentration from 5 to 25 mg/L resulted in increasing SO_4^{2} LC50s (r^2 0.8503, *p* 0.0258) for *H. azteca* (Fig. 1A), although for *C. dubia*, the slope was not signiÞcantly different from zero over this chloride concentration range (r^2 0.4906, *p* 0.1877). In addition, the LC50s for *C. dubia*

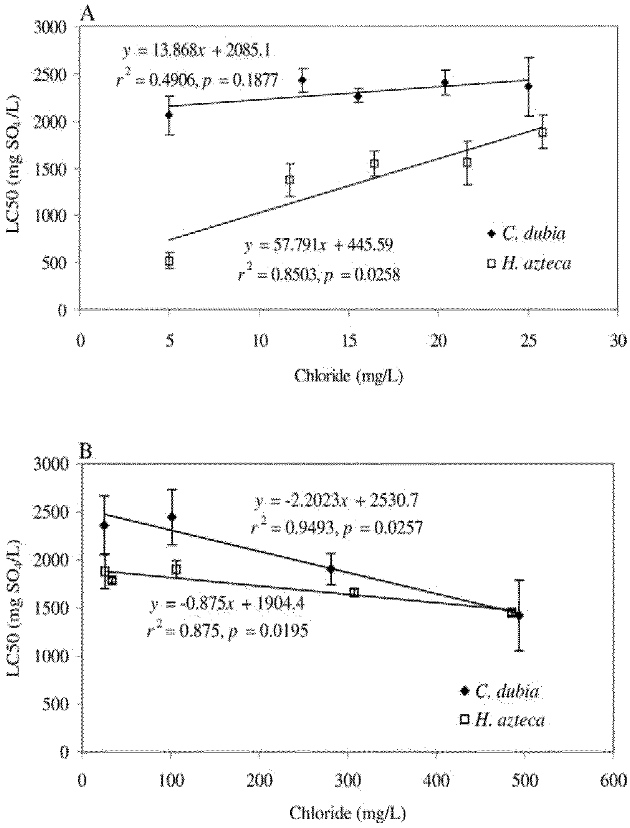

Fig. 1. InÞuence of chloride concentration over two ranges, (**A**) 5 to 25 mg/L and (**B**) 25 to 500 mg/L, on toxicity of sodium sulfate to *Ceriodaphnia dubia* and *Hyalella azteca*. Hardness was approximately 100 mg/L for all tests and Ca:Mg molar ratios were 1.41 except for the tests at 5 mg Cl /L (0.88). LC50 Median lethal concentration.

were higher than those for *H. azteca* for each chloride concentration over this range. When using a combined data set of individual test LC50s for *C. dubia* and *H. azteca* over this chloride range and at hardness 100 (*n* 33), in a simple linear regression analysis with covariance (with species as a treatment effect and chloride concentration as continuous effect), a strong positive relationship was observed (r^2 0.7900, *p* 0.0001) with both the chloride and treatment (species) effects being signiÞcant (*p* 0.0001; Table 1).

Although a positive relationship between chloride concentration and SO_4^{2} LC50 was observed for *H. azteca* over the range of 5 to 25 mg Cl /L, a signiÞcantly negative trend (r^2 0.875, *p* 0.0195) was observed over the range of 25 to 500 mg Cl /L. An even stronger negative relationship (r^2 0.9493, *p* 0.0257) was observed for *C. dubia* over the same chloride range. When using the combined data set of individual test LC50s for *C. dubia* and *H. azteca* over this chloride range and at hardness 100 (*n* 30), in a multiple linear regression analysis with covariance as described above, a negative relationship was observed (r^2 0.6539, *p* 0.0001), with both the chloride and treatment (species) effects being signiÞcant (*p* 0.0001 and *p* 0.0003, respectively; Table 1).

Effects of hardness on toxicity of sulfate to Hyalella *at chloride 25 mg/L*

A strong linear trend of decreased sulfate toxicity with increased hardness (r^2 0.7092, *p* 0.0354) was observed for *H. azteca* (Fig. 2). The LC50 values increased from less than 1,900 mg/L at hardness 100 mg/L, to greater than 4,000

Table 1. Results of multiple regression analysis with covariance for three different subsets of data. Individual median lethal concentrations were used as data points. Data for both species were included in analysis

Term	Estimate	*p*
[Cl] range 5 to 25 mg/L; hardness approximately 100 mg/L		
r^2 0.7900, n 33		
Intercept	1,270.23	0.0001
Chloride	35.14	0.0001
Species	449.68	0.0001
Term	Estimate	*p*
[Cl] range 25 to 500 mg/L; hardness approximately 100 mg/L		
r^2 0.6539, n 30		
Intercept	2,189.48	0.0001
Chloride	1.46	0.0001
Species	178.92	0.0003
Term	Estimate	*p*
Hardness range 100 to 600, Cl 25 mg/L		
r^2 0.5177, n 38		
Intercept	1,969.38	0.0001
Hardness	3.15	0.0001
Species	10.38	0.9046

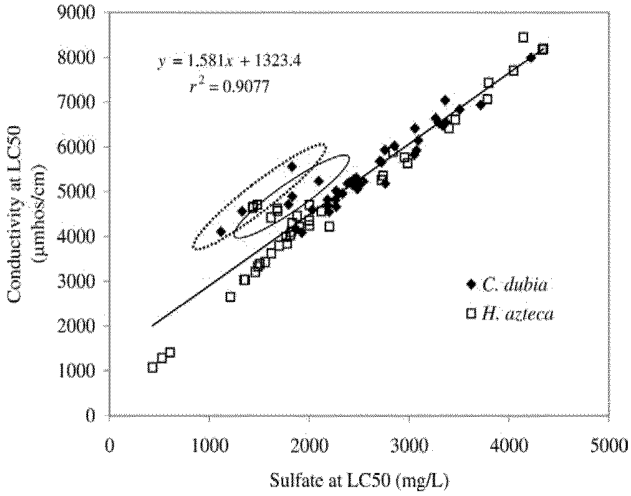

Fig. 3. Relationship between median lethal concentration (LC50) in terms of sulfate (mg/L) and in terms of conductivity (mhos/cm) for tests with *Hyalella azteca* and *Ceriodaphnia dubia*. In addition to the 63 new tests generated for the present study, 19 tests from Soucek et al. [14] with *C. dubia* were included. Hardness values ranged from 100 to 600 mg/L and chloride ranged from 5 to 500 mg/L. Points enclosed by the solid oval were conducted at 300 mg Cl /L and those in the dashed oval at 500 mg Cl /L (nominal concentrations).

mg/L at a hardness of 500 mg/L. The mean LC50 value at 600-mg/L hardness was lower than that at 500-mg/L hardness. It remains unclear how the trend will continue with increasing hardness above 600 mg/L. When using the combined data set of individual test LC50s for *C. dubia* and *H. azteca* over this hardness range (n 38) in a multiple linear regression analysis with covariance as described above, a positive relationship was observed (r^2 0.5177, p 0.0001; Table 1). The hardness effect was observed to be signiÞcant (p 0.0001), but the treatment (species) effect was not (p 0.9046; Table 1).

Relationship between sulfate LC50s and conductivity LC50s

Conductivity LC50s ranged from 1,071 to 8,449 mhos/cm, and LC50s based on sulfate ranged from 512 to 4,345 mg SO_4^{2} /L (Fig. 3). When including all data from the present study and *C. dubia* data from Soucek et al. [14], there was a strong positive relationship between sulfate LC50s and conductivity

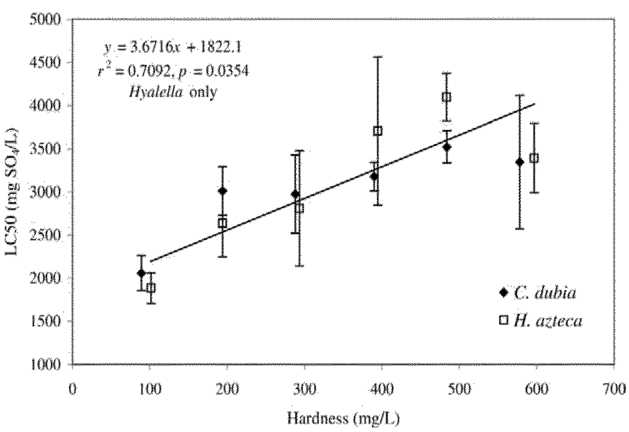

Fig. 2. Inßuence of hardness on toxicity of sulfate to *Hyalella azteca* and *Ceriodaphnia dubia*. The *C. dubia* data are from Soucek et al. [14]. Concentration for all *H. azteca* tests was approximately 25 mg/L, and Ca:Mg molar ratio was 1.41. LC50 Median lethal concentration.

LC50s (r^2 0.9077, p 0.0001; Fig. 3). Twelve data points enclosed in solid and dashed ovals in Figure 3 diverged from the line formed by the remaining points, suggesting sulfate LC50s were lower than would be predicted by conductivity LC50s. These points were for tests with *C. dubia* and *H. azteca* when nominal chloride concentrations were 300 mg/L (solid oval) and 500 mg/L (dashed oval).

Comparison of test results with STR model predictions

All ion concentrations used as input for the STR model were concentrations at the observed mean sulfate LC50 levels for each test solution type (Cl x, hardness y, and Ca:Mg z), so observed percent survival in each case was 50%. For tests with *C. dubia* in which hardness was Þxed at approximately 100 mg/L and chloride varied from 5 to 500 mg/L, the STR model predicted % survival values ranging from 69.0 to 48.4% (Fig. 4A). Most predictions were greater than 50%, and thus the model slightly underpredicted toxicity in most cases.

For tests with *C. dubia* in which chloride was Þxed at approximately 25 mg/L and hardness varied from 100 to 600 mg/L, the STR model predictions were highly variable, ranging from 4.1 to 82.9% survival (Fig. 4B). Only the hardness 100 mg/L prediction was greater than 50% (82.9%); for hardness values of 200 to 600 mg/L, toxicity was strongly overpredicted, with % survival predictions of 4.1 to 21.8.

DISCUSSION

Chloride had a strong but variable inßuence on acute sulfate toxicity, depending on the species tested and the concentration range. In multiple linear regression analyses with covariance, including species as a variable, the species term was signiÞcant over both the lower (5 to 25 mg Cl /L) and the higher (25 to 500 mg Cl /L) chloride ranges. The difference between the two species was most striking over the 5- to 25-mg/L chloride range where LC50s for *H. azteca* were strongly positively correlated with chloride concentration and chloride did not affect the response of *C. dubia* (see Fig. 1). *Hyalella* appears

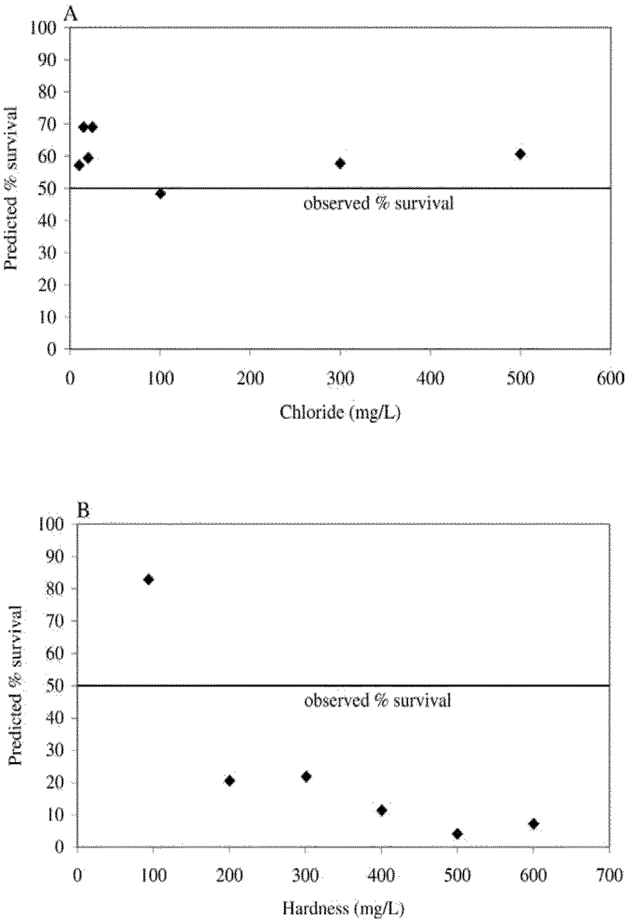

Fig. 4. Percent survival of *Ceriodaphnia dubia* at varying levels of chloride (**A**) and hardness (**B**) as predicted by the salinity/toxicity relationship (STR) model. Points are output predicted by STR and the horizontal line is observed result. Model inputs were ion concentrations at nominal sulfate median lethal concentrations, making observed % survival in each case 50%.

to require a minimal amount of chloride for effective osmoregulation at high sodium sulfate concentrations. Although there are several different osmoregulatory strategies used by freshwater organisms, most freshwater amphipods and daphnid cladocerans regulate hypertonically with respect to the surrounding medium, and this is achieved by active transport of ions into the hemolymph [16–19]. The principal inorganic anion of crustacean hemolymph is chloride, and it has been suggested that low chloride concentrations may limit the distribution of at least one euryhaline amphipod (*Corophium curvispinum*) in freshwaters [20]. Even among amphipods, there is a wide range of sodium and chloride inßux rates and integument permeabilities that determine osmoregulatory effectiveness [20,21]; therefore, it might not be surprising that the responses of *H. azteca* and *C. dubia* to sodium sulfate were quite different over the lower range of chloride concentrations. Although Borgmann [31] suggested that, under low salinity conditions, bromide was required but chloride was not needed by *H. azteca* for survival, growth, and reproduction, data from the present study suggest that the chloride is quite important in determining the effect of elevated levels of sodium sulfate on that species. Laboratory deionized water and concentrated sodium sulfate solutions were analyzed previously for bromide, and levels were below detection limits [26].

Over the higher range of chloride concentrations (25–500 mg/L), a different trend was observed. Although the slopes of the lines for the two crustacean species were different, there was a negative correlation between chloride concentration and sulfate LC50 for both species. The trend was stronger for *C. dubia*, with a more negative slope (2.2) compared to *H. azteca* (0.875), although r^2 values were high and relationships statistically signiÞcant for both. These data suggest that, over this range of chloride concentrations, chloride and sodium sulfate toxicity are additive. Chloride LC50s (as NaCl) for *C. dubia* generally range from 900 to 1,200 mg Cl /L (e.g., [12]), and so the highest two chloride concentrations in the present study were likely to cause some toxicity without sulfate present.

Hardness had a strong inßuence on sulfate acute toxicity that was similar for both crustacean species. A number of studies have provided evidence that increasing hardness ameliorates toxicity of waters with high dissolved solids concentrations [7,9,12,15] and Soucek and Kennedy [14] showed quantitatively that, in a sodium-dominated system, sulfate toxicity to *C. dubia* is reduced as hardness progressively increases, albeit with diminishing returns in the hardness 600 mg/L range. In the present study, the results of multiple linear regression analyses indicated no difference between the sensitivities of the two species over the hardness range of 100 to 600 mg/L as $CaCO_3$. This was in contrast to the results of the tests in which chloride was varied, where the two species had different slopes over both ranges of chloride concentrations examined. In addition, these results are notable because nearly identical slopes were observed for the two species despite the fact that the waters for tests conducted with *C. dubia* had a different chloride concentration (5 mg/L) and Ca:Mg molar ratio (0.88) than those used for tests with *H. azteca* (25 mg Cl /L and 1.41 Ca:Mg molar ratio). As an explanation for this phenomenon of hardness ameliorating sulfate toxicity, Soucek and Kennedy [14] proposed that increased calcium concentrations decrease the passive permeability of epithelial cells to water and ions in various aquatic organisms [32,33], reducing passive diffusion and the energy required to osmoregulate and accounting for the decrease in toxicity. Calcium can mitigate hydrogen ion toxicity to aquatic organisms by decreasing membrane permeability to H and stimulating active Na uptake ([see [34]); however, Potts and Fryer [35] found that calcium had little effect on sodium loss in *Daphnia magna*. Although data from the present study support this hypothesis, other explanations are possible and empirical work is needed to determine the mechanism behind the phenomenon.

In the present study, LC50s in terms of conductivity were highly correlated with LC50s in terms of sulfate for both species, except when extremely high chloride concentrations were used (300 to 500 mg/L). The plots of conductivity LC50s and sulfate LC50s clearly illustrate the contention that knowledge of the contribution of various major ions is critical to effectively managing produced waters or efßuents with high concentrations of dissolved solids [2]. Not only did sulfate LC50s range from 512 to 4,345 mg/L, but conductivity LC50s ranged from 1,071 to 8,449 mhos/cm. These wide ranges were observed for just two species with relatively similar sensitivities. Clearly, any attempt at water quality standard development, whether based on TDS, conductivity, sodium, or sulfate, should incorporate the fact that the water quality parameters like hardness and chloride strongly regulate the toxicity of high TDS solutions. Finally, the conductivity/sulfate plots provide further evidence that chloride and sulfate toxicity are additive. When chloride was less than or equal to 100 mg/L, sulfate toxicity was strictly related to conductivity; however, when 300 and 500 mg Cl /L solutions were tested, sulfate LC50s were lower than predicted by LC50s based on conductivity.

When chloride was variable and hardness Þxed at approximately 100 mg/L, the STR model was relatively accurate in predicting toxicity to *C. dubia*; predicted survival ranged from 48 to 69%, and observed survival was 50% in each case because calculated ion concentrations at observed sulfate LC50 were used as inputs. With one exception (48%), the model underpredicted toxicity for this data range. This might be because the STR model largely is based on the results of fed tests, which the authors acknowledged had a small inßuence on test results [12]. However, Soucek ([36]; http://www.pca.state.mn.us/news/eaw/buffalolake-item23.pdf) compared 48-h sulfate LC50s in unfed tests using moderately hard, reconstituted water [23] and reformulated moderately hard reconstituted water [25] as diluents with 48-h sulfate LC50s obtained from fed, 7-d chronic tests in the same two diluents. In both cases, average LC50s for unfed tests were signiÞcantly lower than those in fed tests. This factor alone might explain the discrepancy between predicted and observed results for *C. dubia* for these tests.

When chloride was held constant (5 mg/L) and hardness was varied from 100 to 600 mg/L, the STR model was relatively inaccurate in predicting toxicity to *C. dubia*, with a trend of underprediction at hardness 100 mg/L, followed by increasing degrees of overprediction at hardness 200 to 600 mg/L. This Þnding is in agreement with Kennedy et al. [15], who found that the STR model overpredicted toxicity to *C. dubia* in sodium sulfate–dominated coal-processing efßuents with hardness values in the 700- to 800-mg/L range. The present study suggests that the STR model does not account for the protective effect of hardness on major ion/TDS toxicity; however, because of the presence of a pattern in the inaccuracy, data from the present study might be useful in improving the model.

CONCLUSION

In conclusion, chloride had a strong but variable inßuence on the acute toxicity of sulfate, depending on the species tested and the concentration range. Over the 5- to 25-mg/L chloride range, mortality of *H. azteca* decreased with increased chloride concentration and chloride did not affect the response of *C. dubia*. The opposite trend was observed over the higher range of chloride concentrations (25–500 mg/L) where increasing chloride concentrations resulted in increased mortality at given sulfate concentrations for both species. Hardness had a strong inßuence on sulfate acute toxicity that was similar for both crustacean species, and nearly identical LC50/hardness slopes were observed for the two species despite the fact that test waters for the two species had different chloride concentrations and Ca:Mg ratios. The LC50s in terms of conductivity were highly correlated with LC50s in terms of sulfate for both species. The wide range of values for both conductivity and sulfate LC50s suggests that single-value water quality standards for TDS, conductivity, sodium, or sulfate are not practical, and the fact that water quality parameters like hardness and chloride strongly regulate the toxicity of high TDS solutions should be incorporated into standard development. In addition, both the sulfate LC50/chloride plots and the conductivity/sulfate plots provided evidence that chloride and sulfate toxicity are additive. The STR model predicted toxicity to *C. dubia* relatively accurately when chloride was variable and hardness Þxed at approximately 100 mg/L; however, the model does not account for the protective effect of hardness on major ion/TDS toxicity. Data from the present study would be a useful incorporation to the STR model.

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